Application of the pH-Stat for the Measurement of Proteolysis

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The methods ordinarily used to follow the opening of peptide bonds in proteins often fail in cases of limited proteolysis due to lack of sensitivity. Provided that the enzymatic reaction takes place at pH-values which are so high that most of the liberated amino groups exist in the unionized form, it is, however, possible to follow the opening of the peptide bonds by continuous titration at a constant pH by means of a Jacobsen-Léonis pH-stat. Different constructions of this device will be discussed. Using the pH-stat it is possible at pH 8 to follow the opening of a single peptide bond in the conversion of ovalbumin to plakalbumin. It has been attempted to correlate the opening of this bond with the change in solubility and crystal form. It was further observed that the salt-free ovalbumin solutions need a small continuous addition of alkali in order to keep pH constant, and the rate of this alkali addition seems to depend on the pH at which the ovalbumin was stored previously to the experiment.

Studies on a Myosin Fragment Obtained by Ammonium Sulphate Treatment of Myosin

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Some preliminary data about the fragmentation of myosin with neutral ammonium sulphate have been given in another communication [1].

By treatment of the myosin with ammonium sulphate at 20° C two different fragments are obtained in nearly equal amounts by weight. Further studies have now been performed on the water-soluble fragment (H_{20}). This fraction precipitates in the preparation process between 40—50 % saturation of ammonium sulphate but cannot, after having been redissolved, be precipitated again with ammonium sulphate. It does not show any ability to associate with actin. The fragment is unstable and with time it is transformed to a large extent into dialysable pieces. During the deterioration process many different ultraviolet spectra can be obtained by different treatments. The fraction seems at first to break into three different parts as indicated by paper-chromatographic analysis. The final product obtained is dialyzable and has a high positive charge. Amino acids can no longer be obtained from it by acid hydrolysis.

Different means have been tried to stop the deterioration of the H_{20} fragment when it just has been obtained by the ammonium sulphate treatment. It has hereby been found that a treatment of the newly formed fragment with calcium hydroxide can stop the deterioration and a product is obtained which has the ability to associate with actin with an ensuing viscosity increase. This actin complex responses to ATP with a viscosity decrease independent of the salt concentration, and the viscosity increases again after a certain time to the normal level. A treatment with potassium oxalate of the calcium hydroxide-treated H_{20} fragment does not alter this effect. If the calcium hydroxide is added in a later step when the deterioration has begun, no effect of it will be found. As yet no other means have been found to restore the activity. The protein treated with calcium hydroxide shows an UV absorption spectrum with a maximum at 260—270 mμ.

From different experiments it is quite obvious that the myosin molecule must possess a unique structure. Probably some compound containing calcium is of importance in the reactions of the myosin, and when the calcium ions are removed this compound is very labile.

1. Snellman, O. *Biochim. et Biophys. Acta (In press).*

Studies on Human Acute Phase Protein (C-reactive Protein)

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The fractionation of a sample containing sera from two persons in the acute phase of streptococcal pharyngitis and maxillary sinusitis, respectively, has been reported by Hed-